EFFECT OF ATRIPLEX FARINOSA, ATRIPLEX NUMMULARIA AND FICUS INGENS ON ULCERATIVE COLITIS IN RATS

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ABSTRACT

The ethanolic extracts of Atriplex farinosa, Atriplex nummularia and Ficus ingens were evaluated for their potential anti-ulcerogenic activity, in addition to total phenolic content and antioxidant activity. Acute and sub-chronic toxicities of the tested extracts were evaluated in mice and rats respectively. In the ulcerative colitis study, extracts were administered orally (200 and 400 mg/kg) to rats once a day for 5 consecutive days and the last dose was administered 2 h before induction of colitis by intra-rectal acetic acid infusion. The inflammatory response was assessed by macroscopic scoring and estimation of myeloperoxidase (MPO) activity. It was noticed that oral pretreatment with the F. ingens (200, 400 mg/kg), A. farinosa, A. nummularia (400 mg/kg) extracts for 5 days before induction of colitis, protected against colonic ulceration and MPO activity elevation. Results showed a valuable effect of F. ingens, A. farinosa and A. nummularia extracts against acetic acid-induced ulcerative colitis.

Keywords: Atriplex farinosa, Atriplex nummularia and Ficus ingens, Ulcerative Colitis, Ulcer Index
INTRODUCTION
Genus *Atriplex* comprises about 200 species belonging to family Chenopodiaceae. *Atriplex farinosa* is a tall shrub of yellowish white appearance with large, naked panicles, but leaf base are cordate with long, obtuse auricles, fruit bracts are entire, longer than broad, acute [1]. Some reports suggested the presence of naringin, naringenin 7-O-glucoside, isorhamnetin-3-O-rhamnosyl (1-6) glucopyranoside and isorhamnetin-7-O-glucopyranoside in *A. farinosa* [2]. The sublethal concentrations of *Atriplex halimus* L. extract reduced mortality rate, longevity, egg production and egg hatchability of *Biomphalaria alexandrina*. This reduction was correlated to the increase in plant concentration [3]. Serum alanine aminotransferase, aspartate aminotransferase, blood urea and serum creatinine were increased in sheep fed on *Atriplex lentiformis* for 45 days, also reduction in live body weight occurred [4]. *A. halimus* produce polyphenols and other bioactive substances potentially useful for medicinal properties and as natural food preservation [5].

*Ficus* is a genus belonging to family Moraceae, it comprises about 850 species of woody trees, shrubs, vines, epiphytes, and hemiepiphyte. *F. ingens* is an evergreen deciduous tree up to 10 m height, occasionally higher, with a rounded or spreading crown and with a spread of up to 30 m wide [6].

Many active compounds were isolated from *Ficus benghalensis* bark; 20-tetratriacontene-2-one, 6 heptatriacontene-10-one, pentatriacontan-5-one, β-sitosterol, β-D-glucoside and meso inositol [7]. In addition, the fruit extract of *F. benghalensis* exhibited antitumor activity [8], while the methanol extract of *F. benghalensis* possess antioxidant activity *F. sycomorus* extracts are used in folk medicine in the treatment of infertility and sterility in humans. *F. capensis* extract was used for treatment of a zoospermia. *Ficusas perifolia* extract has been reported to have an estrogenic effect in female rats [9].

*F. ingens* offered eight compounds which identified as α-sitosterol, α-sitosterolglucoside, chryasophanol, 7-hydroxy-2, 5-dimethyl chromen-4-one, quercetin, Aloe emodinglucoside, rutin and patuletin-3-O-methyl-3-O-rutinoside, also significant protection against CCl₄ hepatotoxicity in rats, which may be attributed to its phytochemical constituents (which were mainly polyphenolic compounds) with their antioxidant and membrane stabilizing properties.
Ethanolic extract of *F. benghalensis* (100 and 200 mg/kg) showed antiulcer effect, supporting the claim that it is protective for gastrointestinal tract. The dose 200 mg/kg of the extract showed more potent antiulcer activity than the lower dose level [11].

**MATERIALS AND METHODS**

**Plant Material**

*Ficus ingens* was collected from Tabuk area, Saudi Arabia during spring 2010, *Atriplex farinosa* was collected from Eastern Desert of Egypt during summer 2010, while *Atriplex nummularia* was collected from North Western Desert of Egypt during summer 2010. The collected plant samples were kindly identified by Dr. Ahmed Morsy Ahmed, Prof. of plant ecophysiology, Desert Research Center, Egypt. A voucher specimen has been deposited in the herbarium of Chemistry Department, Faculty of Sciences, King Saud University. Plant material was shade-dried, reduced to fine powder, packed in tightly closed containers and stored at room temperature for phytochemical and biological studies.

**Extraction**

About 750 g of the dried powder of the aerial parts of each plant was percolated in 70% aqueous ethanol with occasional shaking for 24 hours. The process was repeated for three times. The combined ethanolic extracts were concentrated under reduced pressure at a temperature not exceeding 35°C.

**Determination of Total Phenolic and Flavonoid Contents**

Total phenolic content of the tested extracts was determined according to Folin-Ciocalteu method [12], using gallic acid as standard. The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. Total phenolic content was expressed as gallic acid equivalents (GAE) per mg of extract. Total flavonoid content was determined by a colorimetric method of [13] and calculated using a quercetin calibration curve. The results were expressed as quercetin equivalents (QE) per mg of extract.

**Antioxidant Activity**

The free radical scavenging activity of the ethanolic extracts of *A. farinosa*, *A. nummularia* and *F. ingens* was determined with the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay using Trolox (2.5 mM in methanol) as a reference substance [14]. The results (Mean ± SD of at least three measurements) were expressed as Trolox equivalent antioxidant capacity (TEAC).

\[
\% \text{ Inhibition of DPPH Activity} = \frac{A-B}{A} \times 100
\]

Where A is the optical density of the blank and B is the optical density of the sample.

**Animals**
Male Wistar albino rats weighing 180–200 g were used for the study. Animals were maintained under standard temperature (22±1°C), relative humidity (55±10%), and 12h light: 12h dark cycle, and fed a standard pellet diet with water ad libitum. They were housed in standard polypropylene cages with wire mesh top. All studies were carried out using six animals in each group. The care and handling of the animals were in accordance with the internationally accepted standard guidelines. All animal procedures were approved by an institutional review board of Pharmacy College, Salman Bin Abdulaziz University, KSA.

Acute toxicity and determination of median lethal dose (LD50)
LD50 of the ethanol extracts of A. nummularia, A. farinosa and F. ingens were determined in rats according to the method of [15]. Male Wistar albino rats in groups of six, received one of 1000, 2000, or 4000 mg/kg of the tested extracts. Control animals received the vehicle and kept under the same conditions. Signs of acute toxicity and number of deaths per dose within 24 h were recorded and the LD50 was calculated as the geometric mean of the dose that resulted in 100% mortality and that which caused no lethality at all.

Measurement of Liver and Kidney Function Markers
Liver functions were evaluated by measuring the serum activity of alanine transaminase (ALT), aspartate transaminase (AST), following the method of [16]. The serum concentrations of total bilirubin (TB) [17], total protein (TP) and albumin (Alb) [18] were estimated. Serum levels of urea [19] and creatinine [20] were determined colorimetrically as measures of kidney functions.

Effect on Ulcerative Colitis
The total ethanolic extract of A. nummularia, A. farinosa and F. ingens and the standard; dexamethasone (DEX) were suspended separately in 3% v/v Tween 80 (vehicle). Fifty four Wistar albino rats were divided into 9 equal groups: Groups 1and 2 (normal and colitis control groups, respectively) were given the vehicle in a dose of 5mL/kg. Group 3 (reference group) was given DEX in a dose of 0.2 mg/kg. Groups 4 and 5 were administered the ethanolic extract of A. nummularia in doses of 200 and 400 mg/kg, respectively. Rats of groups 6 and 7 were medicated with the ethanolic extract of A. farinosa (200 and 400 mg/kg, respectively. Groups 8 and 9 were treated with F. ingens extract in doses of 200 and 400 mg/kg, respectively. All medications were...
administered orally via the aid of an orogastric cannula, once daily for 5 consecutive days and the last dose was administered 2 h before colitis induction.

**Induction of Ulcerative Colitis**

Rats were fasted for 24 h with access to water *ad libitum* after which they were lightly anesthetized with ether. A polyethylene catheter with 2 mm diameter was inserted through the rectum into the colon to a distance of 8 cm [21]. For Ulcerative colitis induction, 2 mL of 4% (v/v) acetic acid was infused into the colon of all rats (except the normal control group) through the catheter, held in place for 30 sec, and then flushed with 5 mL of phosphate buffer solution; pH=7. The catheter was left in place for few seconds then gently removed.

**Assessment of Colonic Lesions**

Two days after the induction of colitis, each rat was inspected and diarrhea was recorded. Rats were sacrificed using ether anesthesia and colonic segments (8 cm in length and 3 cm proximal to the anus) were excised, opened along its mesenteric border, and rinsed thoroughly in ice-cold normal saline. The colon specimen of each rat was weighted and wet weight/length ratio was calculated as ratio of the colon specimen weight vs its length (mg/cm). It was used as a parameter to assess the degree of colon edema, which reflected the severity of colitis. The specimens were examined under a dissecting microscope and all visible damages were evaluated using the scoring system reported by [22] with some modifications. The lesion scores were: 0 = no damage, 1 = Local edema and inflammation without ulcers; 2 = One ulcer without inflammation; 3 = one to two ulcers with inflammation and lesion diameter < 1 cm; 4 = More than two ulcers with lesion diameter 1-2 cm; 5 = Sever ulceration with lesion diameter >2 cm. Ulcer area was measured for each specimen using a 1-mm² grid. Ulcer index was measured by summing the lesion score and the ulcer area for each colon specimen [23].

**Effect on the Activity of Myeloperoxidase (MPO) in the Colonic Mucosa of Rats**

The colonic mucosa was carefully scraped off from the colon of each rat with a glass slide and snap-frozen in liquid nitrogen, and stored at −80 °C for the assay of MPO activity within a week. The frozen colonic mucosa was homogenized with a homogenizer in phosphate buffered saline then MPO activity was determined by a modified method described by [24].

**Statistical Analysis**

All the values were expressed as mean ± S. E. M. Statistical analysis was done by using SPSS 10 and statistical significance of
differences between two means was assessed by unpaired Student’s ‘t’ test. Differences at P ≤ 0.05 were considered statistically significant.

RESULTS

Determination of Total Phenolic and Flavonoid Contents

The present results (Table 1) showed that the ethanolic extracts of *A. nummularia*, *A. farinosa* and *F. ingens* have high total phenolic content (144.4±6.11, 141.3±5.20 and 166.3±6.20 mg GAE per g extract, respectively). *F. ingens* was found to have the highest total flavonoid content (98.0±3.32, while *A. farinosa* displayed lower total flavonoid content (80.0±2.10 mg QE per g extract).

Antioxidant Activity

In the present study *A. nummularia*, *A. farinose* and *F. ingens* extracts showed antioxidant and DPPH radical scavenging activities (Table 1). The highest antioxidant activity was shown by the ethanolic extract of *F. ingens* (1.88 TEAC). The antioxidant activity of *A. nummularia* (1.71 TEAC) and *A. farinosa* (1.71 TEAC).

Acute Toxicity and Determination of LD50

The obtained results indicated that different doses of *A. nummularia*, *A. farinosa* and *F. ingens* extracts (1000, 2000 and 4000 mg/kg b.wt.) did not produce any symptom of acute toxicity and none of the rats died during 24 h of observation. All rats did not exhibit diarrhea, haematuria, restlessness, uncoordinated muscle movements, and respiratory distress. Accordingly, it suggested that oral LD50 of the tested extracts were higher than 4000 mg/kg b.wt.

Sub-Chronic Toxicity

In the present study, oral dosing of the tested extracts to rats in doses of 200 and 400 mg/kg for 35 days did not show any significant effect on the levels of ALT, AST, total bilirubin, total proteins, and albumin in their sera as compared to control. No significant change in the mean values of urea and creatinine was estimated in sera of rats following 35 days of extracts administration at doses of 200 and 400 mg/kg when compared with the control.

Anti-Ulcerogenic Effect

In the present study, no abnormal changes were observed in rats of the normal control group suggesting that handling procedure had no interference with the experimental outputs. Experimental colitis was accompanied by marked anorexia, prostration, hypo motility and pilorection after acetic acid challenge (data not shown). Diarrhea, as evidenced indirectly by perianal fur soiling, was prominent among colitic animals. The incidence of diarrhea, lesion score, ulcer area
and ulcer index were used as the indicators for the effectiveness of the tested extracts against colitis induced by acetic acid in rats (Table 2). Rats of the control colitis group developed severe diarrhea (100%) after rectal acetic acid infusion. DEX and the ethanolic extracts of *A. nummularia, A. farinosa* and *F. ingens* at the high dose (400 mg/kg) reduced the incidence of diarrhea to 15.9, 50.0, 39.4 and 34.2%, respectively and the diarrhea was much milder. The low dose of the tested extracts (200 mg/kg) had weak effect in protecting against diarrhea of the rats with colitis.

**Effect on the Activity of Myeloperoxidase (MPO) in the Colonic Mucosa of Rats**

MPO activity indicates the degree of inflammatory cell infiltration, which is a marker of acute inflammation. In this study, the activity of MPO was assessed in the colonic mucosa of all tested rats and the results are shown in Table 2 and Figure 1. The myeloperoxidase assay indicates that the activity of MPO was low in the normal control rats (0.17 ± 0.02 U/g). The activity of MPO elevated in rats with acetic acid colitis (11.168 ± 1.3 U/g). The ethanolic extracts of *A. nummularia, A. farinosa* at the dose of 400 mg/kg significantly inhibited the elevation of MPO activity in the rats with colitis. The activity was decreased to 6.88 ± 0.61; 8.11± 0.66 respectively, and in the case of *F. ingens* the activity was decreased to 8.55± 0.9 and 5.63 ± 0.33 U/g at the dose of 200 and 400 mg/kg respectively, the effect of DEX in inhibiting the activity of MPO (4.62 ± 0.43 U/g).

**DISCUSSION**

**Determination of Total Phenolic and Flavonoid Contents**

The ethanolic extracts were tested phytochemically to ensure the presence of polyphenols and flavonoids. Our results showed that the ethanolic extracts of *L A. nummularia, A. farinose* and *F. ingens* have high total phenolic and flavonoid contents. Phenolic compounds are secondary metabolites which synthesize in plants. They possess some biological properties such as: antioxidant, anti-apoptosis, anti-aging, and anti-inflammation. Flavonoids are a large group of ubiquitous molecules synthesized by plants. Many studies have shown that flavonoids play important pharmacological roles against various human diseases, such as cardiovascular diseases, cancer, inflammation and allergies [25].

**Antioxidant Activity**

The model DPPH provides a method to evaluate antioxidant activity in a relatively short time compared to the other methods. The antioxidant activity of the tested plants
could be attributed to their total phenolic and flavonoidal contents. There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups [26]. It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidants. In addition, flavonoids act as scavengers of various oxidizing species i.e. super oxide anion, hydroxyl radical or peroxyl radicals, they also act as quenchers of singlet oxygen [27].

**Biological Studies**

All rats treated with different doses of *A. nummularia*, *A. farinose* and *F. ingens* extracts were alive during 24 h of observation. It suggested that the LD$_{50}$ of these extracts was higher than 4000 mg/kg. Therefore, the tested plants can be categorized as highly safe since substances possessing LD$_{50}$ higher than 50 mg/kg are nontoxic [28].

**Sub-Chronic Toxicity**

The non toxic nature of the ethanolic extracts of *A. nummularia*, *A. farinose* and *F. ingens* in acute toxicity study is well supported by the normal levels of biochemical data (ALT, AST, total bilirubin, total proteins and albumin) following 35-days treatment period in rats. The serum transaminase level is most widely used as a measure of hepatic injury, due to its ease of measurement and high degree of sensitivity. It is useful for the detection of early damage of hepatic tissue. Since the activity of ALT and AST are specific assayable liver enzymes, their normal levels in serum of experimental groups of rats treated for 35 days means that the three tested plants are not hepatotoxic.

Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. In kidney damage, there will be retention of urea and creatinine in the blood, therefore marked increase in serum urea and creatinine are indications of functional damage to the kidney [29]. By these indicators, ethanolic extracts of *A. nummularia*, *A. farinose* and *F. ingens* are therefore, not nephrotoxic in rats.

**Anti-Ulcerogenic Effect**

The model of acetic acid induced colitis shares many of the histological features of UC in human beings including mucosal edema and submucosal ulceration [30]. The protective effect of the tested extracts against acetic acid induced ulcers could be attributed to their phenolic and/or flavonoid content and their reactive oxygen species scavenging property. The antioxidative mechanism of the tested extracts against colon mucosal lesions was supported by their *in vitro* antioxidant potency.MPO was found predominantly in
neutrophils, monocytes, and macrophages [31] and has been implicated as a participant in tissue injury during inflammatory diseases [32]. Several reports have demonstrated increased neutrophil infiltration in inflammatory mucosa [33]. Such infiltration might be regarded as a trigger of free radicals release. Increased production of free radicals and impaired antioxidant defense mechanisms are postulated to be causative factors in inflammatory diseases. Accordingly, estimation of MPO activity in colonic mucosa has been used as an indicator of neutrophil influx into inflamed colon tissue [34]. In the present investigation, the ethanolic extracts of A. nummularia, A. farinose and F. ingens at the dose of 400 mg/kg attenuated colon mucosal damage and subsequently reduced MPO activity in colonic tissues. Treatment with F. bengalensis significantly declined scores indices and decreased the MPO activity. This protective effect of F. ingens may be attributed to the high flavonoid content of this plant, in addition to its antioxidant activity.

CONCLUSION
The tested ethanolic extracts were well tolerated following acute and sub-chronic treatment and they neither produced overt signs of clinical toxicity nor any signs of hepato- or nephro-toxicity. Moreover, the ethanolic extracts attenuated the macroscopic colonic damage -induced by acetic acid and inhibited the elevation of MPO activity in the colonic mucosa in rats with colitis. These results suggest that the ethanolic extracts of A. nummularia, A. farinose and F. ingens may be effective in the prevention of UC through their anti-inflammatory and scavenging effect on oxygen-derived free radicals. However, more detailed phytochemical studies are necessary to identify the active principles and exact mechanism of action.

ACKNOWLEDGMENT
The authors expressed their deepest thanks to the Deanship of Scientific Research at Salman Bin Abdulaziz University, Al-Kharj, KSA, for the work through the research project No. 10/Ph/1432.

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Table 1: Total Phenolics, Total Flavonoids and Antioxidant Activity of the Tested Plant Extracts

<table>
<thead>
<tr>
<th>Plant</th>
<th>Total phenolics (mg GAE per g extract)</th>
<th>Total flavonoids (mg QE per g extract)</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TEAC%</td>
</tr>
<tr>
<td>A. farinosa</td>
<td>141.3 ±5.20</td>
<td>80.0 ±2.10</td>
<td>1.70 ±0.06</td>
</tr>
<tr>
<td>A. nummularia</td>
<td>144.4 ±6.11</td>
<td>81.0 ±2.33</td>
<td>1.71 ±0.10</td>
</tr>
<tr>
<td>F. ingens</td>
<td>166.3 ±6.20</td>
<td>98.0 ±3.32</td>
<td>1.88 ±0.09</td>
</tr>
</tbody>
</table>

NOTE: GAE = Gallic acid equivalent, QE = Quercetin Equivalent, TEAC = Trolox Equivalent Antioxidant Capacity

Table 2: Effects of Oral Administration of Ethanolic Extract of Atriplex farinosa on the Macroscopic Parameters of Ulcerative Colitis Induced by Acetic Acid in Rats (n = 6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Diarrhea (% incidence)</th>
<th>Lesion score (0-5)</th>
<th>Ulcer area (cm²)</th>
<th>Ulcer index</th>
<th>MPO U/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>00</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>Colitis control</td>
<td>00</td>
<td>100.0</td>
<td>3.7 ± 0.18</td>
<td>5.2 ± 0.26</td>
<td>8.9 ± 0.35</td>
<td>11.16±1.30</td>
</tr>
<tr>
<td>DEX</td>
<td>0.2</td>
<td>15.9</td>
<td>15.4</td>
<td>2.12±0.14*</td>
<td>2.16±0.15*</td>
<td>4.62±0.43</td>
</tr>
<tr>
<td>Atriplex farinosa</td>
<td>200</td>
<td>83.3</td>
<td>3.5 ± 0.21</td>
<td>4.9 ± 0.20</td>
<td>8.4 ± 0.40</td>
<td>10.15±1.20</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>33.3</td>
<td>2.1 ± 0.20*</td>
<td>4.0 ± 0.14*</td>
<td>7.1 ± 0.35*</td>
<td>8.11±0.66*</td>
</tr>
<tr>
<td>Atriplex nummularia</td>
<td>200</td>
<td>83.3</td>
<td>3.6 ± 0.17</td>
<td>4.9 ± 0.24</td>
<td>8.7 ± 0.37</td>
<td>10.11±1.00</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>50.0</td>
<td>2.9 ± 0.13*</td>
<td>3.6 ± 0.204*</td>
<td>6.3 ± 0.28*</td>
<td>6.88±0.61*</td>
</tr>
<tr>
<td>Ficus ingens</td>
<td>200</td>
<td>66.7</td>
<td>2.6 ± 0.25*</td>
<td>3.4 ± 0.38*</td>
<td>6.1 ± 0.52*</td>
<td>8.55±0.90*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>33.3</td>
<td>2.9 ± 0.21*</td>
<td>3.2 ± 0.31*</td>
<td>5.9 ± 0.50*</td>
<td>5.63±0.33*</td>
</tr>
</tbody>
</table>

NOTE: Significant at *P ≤ 0.05

Figure 1: Effect of DEX and Ethanolic Extract (400 mg/kg) of A. farinosa, A. nummularia and F. ingens on MPO Activity of Colonic Mucosa in Rats with Colitis Induced by Acetic Acid